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LONG-TERM CONSEQUENCES OF SUBTLE STIMULI DURING THE FIRST TWENTY-FOUR HOURS OF SEIZURE-INDUCED BRAIN INJURY[1]

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Summary.—Chronically epileptic (induced by a single systemic injection of lithium and pilocarpine about 30 days before the experiment began) male rats were trained within a radial maze while they were administered either GABA-pentin (Neurontin), or prednisolone or given no treatment. There was no significant improvement in learning or memory between the groups. Numbers of trials per day were positively correlated with the time required to display the overt stereotyped forelimb clonus after the single pilocarpine injection. The numbers of correct trials completed during the first few days of acquisition were significantly greater for the rats that had received weak (1 μT) complex, pulsed magnetic fields over the right hemisphere during the first 24 hr. after seizure induction than for those who received the same field over the left hemisphere or that had been exposed to reference conditions. Implications of the enhanced sensitivity of limbic neurons to subtle electromagnetic interaction during electrical lability are discussed.

Approximately 80% of epileptic patients whose seizures are due to limbic anomalies still exhibit electrical anomalies despite medication by traditional anticonvulsant drugs (Heinemann, Zhang, & Edler, 1994). A closely congruent animal model of limbic epilepsy can be induced following a single, subcutaneous injection of lithium followed a few hours later by a single, subcutaneous injection of pilocarpine (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993). The rats become chronically epileptic, as defined by occasional, spontaneous limbic automatisms. They are defined as sequences of ear-twitches and vibrissae movements that are followed by rearing, rapid forelimb clonus, and falling; about 30 sec. later the rats become hyperresponsive (fearful) to stimuli for about 0.5 to 2 min.

Recent pharmacological (Heinemann, *et al.*, 1994) and theoretical developments (Anninos, Vartzopoulos, Razis, & Anogianakis, 1989; Sandyk, 1992) suggested that traditional anticonvulsants may not be optimal for limbic seizures whose foci involve the hippocampal, amygdaloid or entorhinal regions. The present experiment was designed to discern the effects of a low dosage of GABA-pentin (Neurontin) and prednisolone upon the acquisition of a radial maze task that chronically epileptic rats rarely acquire. The first drug was selected because it is an analogue of gamma amino butyric acid (GABA)

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while the second could dampen the erratic release of the epileptogenic neuropeptide corticotrophin releasing factor. Prednisolone has been shown to ameliorate the marked intragroup male aggression that often evolves with this form of limbic epilepsy (Bedard & Persinger, 1995). Relatively low effective doses were implemented to minimize the contribution from sedation or appetitive, e.g., reduction of food-intake, variables.

The second purpose of this study was to discern whether subtle electromagnetic stimuli applied during the first twenty-four hours of the seizure induction can produce long-term changes in the animal's behaviour. This prediction is derived from the enhanced vulnerability of neurons to the normal representations of experience (which are transformed into neuroelectrical patterns) if there has been pre-exposure to glucocorticoids (McEwin, 1994). Other studies from this laboratory have indicated that sensory modality-specific enhancement of damage within the thalamus occurs if that modality is stimulated during the first 24 to 48 hr. after the seizure induction. Although the observations were retrospective, we have measured increased (about 25%) neuronal dropout within the posterior nuclei of the ventral tier of the thalamus if the rats were tail bled (associated with thermal and cutaneous stimulation of the tail) and greater necrosis within the suprageniculate nucleus in groups of rats that were exposed, inadvertently, to unexpected, brief (a few seconds) noise whose amplitudes (about 80 db) were sufficient to induce startle in the normal rat.

If the postseizure brain was that vulnerable, we predicted that even subtle but biogenically, congruent, external electromagnetic fields should induce changes whose consequences would be permanent. In this study we decided to apply a magnetic field pattern that has been associated with enhanced nociceptive thresholds (Fleming, Persinger, & Koren, 1994) by a mechanism that may involve some of the receptor subtypes by which morphine induces analgesia. Our previous work (Bureau & Persinger, 1995) indicated that the daily pairing of exposures of brief periods of complex, pulsed magnetic fields with injections of subclinical dosages of pilocarpine reduced the incidence of subsequent overt seizures. Similarly, exposures to pulsed magnetic fields after experimentally induced ischemia, have been shown to reduce (but not to eliminate) the magnitude of the neuronal necrosis (Grant, Cadossi, & Steinberg, 1994). Because learning of a complex, radial maze should require more right-hemispheric processing (particularly during the earlier stages of learning) a consistent prediction would be a relative enhancement of acquisition by chronically epileptic rats that have received pulsed fields over the right hemisphere relative to the left side.

Method

A total of 12 male albino rats, 100 days of age, were selected as sub-

jects. The rats had been injected subcutaneously with lithium/pilocarpine (3 mEq/kg) and then 4 hr. later by 30 mg/kg of pilocarpine. They were immediately placed in commercial physical restraint cages until the overt seizure, conspicuously evident, occurred and was recorded to the nearest second. Sixty min. after the injection of the pilocarpine the rats were injected subcutaneously with 30 mg/kg of acepromazine, a procedure that facilitates survival (Persinger, *et al.*, 1993).

Four of the rats had been exposed during the first 24 hr. after the seizure induction to a burst firing magnetic field for 1 sec. every 4 sec. (Richards, Persinger, & Koren, 1993) along the right hemisphere while another four had been exposed to the same pattern along the left hemisphere. The fields were delivered by one set of small solenoids (placed adjacently within a plastic capsule) that were arranged to produce a homogeneous field along the caudal-rostral extent of the brain (poles of the two solenoids were in series, i.e., one N, the other S at any given time). The field gradient was 1 microT within the hemisphere adjacent to the solenoids and 0.5 microT (500 nT) within the distal extent of the opposite hemisphere (spatial gradient = 250 nT/cm). The (signal) pattern was delivered through a digital-analog converter that was driven by software from a personal computer. The remaining four rats were exposed to a homogeneous 60-Hz background whose intensity was 100 nT to 150 nT (spatial gradient < 10 nT/cm). The typical background intensity of the 60-Hz range field within the laboratory setting was about 10 nT. At the end of the 24 hr. of exposure to treatments, the rats were removed from the restraint cages, placed into group caging (3 rats/cage), and administered the usual recovery treatment for 10 days (Persinger, *et al.*, 1993).

Thirty days after the seizure induction and treatments, the rats were slowly food deprived to 85% of their free-feeding weight over several days. They were first trained to acquire a lever-press response in a standard operant chamber for two days (50 rewards/day). They were then tested 30 min. per day for eight consecutive days in an automated four-arm radial maze (Persinger, Bureau, & Peredery, 1994) during the midphotophase between 1100 hr. and 1500 hr. For the successful completion of the task, the rat was required to enter each arm, press a lever at the end of the arm, and obtain a food reward. If re-entry into a previously entered and rewarded arm occurred before all of the others had been entered only once, there was no reward. Consequently, the rat had to press the lever for each of the four arms at least once before the next trial (and option for reward) was initiated. The number of completed trials was measured. From the first day of lever training until the last day of maze testing, four of the rats received neurontin in their home-cage food (delivered twice per day) such that the dosage was equivalent to 10 mg/kg or to the approximate human dosage of 700 mg/day

which would be considered a "lower" dosage. Another four rats received 0.25 mg/cc of prednisolone in the water supply during this time while the remaining rats were employed as the reference group. The body weights of the four groups did not differ significantly either before or after the maze training; the grand mean and standard deviation were 365 gm and 39 gm, respectively.

The small number of rats per group for the postseizure exposures and maze training period was considered appropriate because we were interested in large effects that would be sufficient to produce clear alterations in behaviour. A large effect was defined as a treatment whose η^2 (explained variance) was at least 50%. With four animals per group, this effect size would be associated with a probability value of $p < .05$. To accommodate the marked individual differences in the rats' capacity for acquisition learning was measured by dividing each of the scores for Days 3 through 8 by the mean of the numbers of completed trials during the first two days for each rat.

A three-way analysis of variance with one level repeated (improvement ratios) as a function of postseizure-induction treatment (three magnetic field treatments during the first 24 hr. after the seizures had been induced) and drug treatment (during maze training) was completed. The two-way interaction term for the two independent variables was considered inappropriate in light of the sample number per cell; consequently, the degrees of freedom were relinquished to the error term (within-subject variance). Because the latency of seizure onset time is suspected to be associated with the precise pathways of repetitive paroxysmal discharge within the cholinergic synapses (and hence variable excitotoxic damage to specific neuronal groups) before they generalize to overt seizures, this latency was correlated with the later index of learning.

Results

According to the results of the multivariate analysis of variance, there was evidence of learning ($F_{5,35} = 7.70$, $p < .05$) over the trials but no statistically significant interaction over days within either treatments ($F_{10,35} < 1.00$, $p > .05$); however, there was a marked increase in heterogeneity of variance between groups during the latter trials. To test the hypothesis specifically the repeated-measures analysis was completed for Days 3 through 6. The interaction between postseizure (pulsed magnetic field) treatment and days was statistically significant ($F_{4,14} = 12.60$, $p < .05$; $\eta^2 = 70\%$) while the interaction between drugs (during maze acquisition) and days of training was not significant statistically.

Post hoc analysis indicated that, whereas there was no difference in relative improvement for the maze task during Day 3 between the postseizure treatments, the rats that had received the right-hemispheric stimulation ex-

hibited faster learning (M and SD of ratio) during Days 4 ($M=3.8$, $SD=1.3$) and 5 ($M=6.4$, $SD=4.4$) of the training compared to the group that received the fields over the left hemisphere ($M=2.1$, $SD=2.0$ and $M=3.5$, $SD=3.1$, respectively) or the group that served as a reference ($M=2.5$, $SD=1.7$ and $M=3.4$, $SD=0.9$, respectively). There was no significant difference among these three groups with respect to the absolute number of successful trials during the two baseline days ($M=3.2$, $SD=2.2$).

The mean numbers of correct successful completions within the maze for all eight days for all rats was significantly correlated with the latency of onset of the overt seizures more than 30 days previously. To ensure reliability of the relationship Spearman *rho* correlations were completed for rats' performance as a function of their individualized improvement for Days 3 through 8. These Spearman values ($rho > .50$, $p < .05$) were .53, .88 ($p < .001$), .67, .52, .32, and .38, respectively.

Discussion

These results are consistent with our research on humans (Persinger, 1995) and with other research with rodents involving the sudden induction of epilepsy due to catastrophic changes in the cholinergic system by either drugs or mechanical brain injury. Once limbic seizures have been initiated, they are very difficult to terminate and the behavioral anomalies associated with the disruptions in the neural substrate are difficult to compensate. We have not, as yet, successfully isolated treatments that would encourage marked reactive synaptogenesis during learning. Although these rats did not respond to the drug treatments, the fact that the rats improved over eight trials implies learning and hence that a neural substrate of the experience had been established.

A paradigm that applies antiepileptic drugs to brain-damaged, chronic epileptic rats allows the experimenter to explain the possible etiologies for the reduced acquisition. If acquisition does not occur until the implementation of the antiepileptic treatment, then there would be a strong possibility of daily, recurrent interference from subclinical seizures within the hippocampal-amygdaloid complex. Because this change did not occur, but all three groups of chronically epileptic rats still learned (although slowly), the alternative explanation is that brain-damaged animals still learn but at much slower rates. Slowed acquisition rates are also evident in populations of patients who display complex partial epileptic-like seizures following traumatic brain injuries (Persinger, 1995). The reservation to this conclusion is related to drug dosage Our treatment, which would be considered within the "low range" for epileptic patients, may have been too diluted to have been effective.

The observation that the latency of seizure onset, more than a month

before the maze testing, was positively associated with more proficient behaviour and suggests that these rats exhibited less brain damage. A re-examination of other data (Persinger, *et al.*, 1994) also showed a correlation of .40 between maze proficiency and seizure onset times ($n = 14$ rats). When this value was correlated with estimates of neuronal dropout (Bureau, Peredery, & Persinger, 1994) within all structures that exhibited changes within the limits of light microscopy (400 X), strong negative associations were noted between damage in the cingulate region ($rho = -.77$, $p < .001$), anterior hippocampal transition ($rho = -.66$, $p < .01$), and prefrontal cortices (forelimb region, $rho = -.60$, $p < .01$) while positive associations were noted with damage within the dorsal endopiriform nuclei ($rho = .65$, $p < .01$). Because the latter is assumed to be an impedance to the spread of seizures, the maintenance of the electrical perturbations within this region would be expected to produce more local damage which would help spare other regions that ultimately would contribute to the acquisition of spatial memories. Consequently, seizure-onset time, whose range is typically only about 20 min., may represent different patterns of inhibitory collapse that ultimately determine the extent of brain injury and the animal's future behavior.

The other surprising effect, although consistent with our predictions concerning complex, pulsed magnetic fields (Persinger, Richards, & Koren, 1994), was that exposure of the right hemisphere but not the left hemisphere during the first 24 hr. after seizure induction might reduce the damage. Although direct histological measurements were not completed, the relative increase in learning proficiency during the middle portion of the learning curve by the rats that received right-hemispheric stimulation is consistent with our observations that this side of the brain is less affected by the damage that follows the initial induction of the seizure. This inference is based upon the mild but statistically significant decrease in loss of neurons within the right cortical and subcortical structures (with exception of midline thalamic nuclei). In addition, there has been a marked propensity for most subsequent spontaneous occurrences of seizures to either begin or remain lateralized within the right hemisphere (as inferred from the initial onset of left forelimb clonus or rapid, counterclockwise circular rotation).

The general theoretical concept supported by these results is that subtle factors, including electromagnetic patterns, may "embed" their characteristics when complex systems are exhibiting marked plasticity secondary to catastrophic collapse. The general metaphor is that periods of neuroplasticity during which (cellular) genetic systems and synaptic pathways are no longer protected from exogenous stimuli will allow, like an electret, permanent representation of brief, structure-facilitating stimuli.

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